

Claims

1. (Currently Amended) A method for detecting one or more analytes, the method comprising

(a) bringing into contact one or more analyte samples and one or more reporter binding molecules, wherein each reporter binding molecule comprises a specific binding molecule and an amplification target circle, wherein each specific binding molecule interacts with an analyte directly or indirectly,

incubating the analyte samples and the reporter binding molecules under conditions that promote interaction of the specific binding molecules and analytes, and

separating the specific binding molecules that interact with the analytes from the specific binding molecules that do not interact with the analytes,

(b) decoupling the amplification target circles associated with the analytes from the ~~reporter binding molecules~~ specific binding molecules that interact with the analytes,

(c) bringing into contact the amplification target circles and one or more rolling circle replication primers, wherein the amplification target circles each comprise a single-stranded, circular DNA molecule comprising a primer complement portion, wherein the primer complement portion is complementary to at least one of the rolling circle replication primers, and

incubating the rolling circle replication primers and amplification target circles under conditions that promote hybridization between the amplification target circles and the rolling circle replication primers,

(d) incubating the rolling circle replication primers and amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA, wherein detection of tandem sequence DNA indicates the presence of the corresponding analytes.

2. (Original) The method of claim 1, wherein at least one of the reporter binding molecules further comprises a circle capture probe, wherein the amplification target circle of the

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

reporter binding molecule is associated with the reporter binding molecule via a non-covalent interaction with the circle capture probe.

3. (Original) The method of claim 2, wherein the non-covalent interaction is base pairing.
4. (Original) The method of claim 3, wherein decoupling of the amplification target circle is accomplished by disrupting the base pairing.
5. (Original) The method of claim 4, wherein the base pairing is disrupted by heating the reporter binding molecules.
6. (Original) The method of claim 2, wherein the circle capture probe comprises an oligonucleotide.
7. (Original) The method of claim 6, wherein the oligonucleotide cannot be extended.
8. (Original) The method of claim 7, wherein the oligonucleotide comprises a 3' end and a 5' end, wherein only the 5' end is free.
9. (Original) The method of claim 8, wherein the oligonucleotide is coupled to the specific binding molecule of the reporter binding molecule via the 3' end of the oligonucleotide.
10. (Original) The method of claim 8, wherein the 3' end of the oligonucleotide is blocked.
11. (Original) The method of claim 7, wherein the oligonucleotide is blocked.
12. (Original) The method of claim 1, wherein at least one of the reporter binding molecules further comprises a circle linker, wherein the amplification target circle of the reporter binding molecule is coupled to the reporter binding molecule via the circle linker.
13. (Original) The method of claim 12, wherein the circle linker comprises a cleavable bond.
14. (Original) The method of claim 13, wherein decoupling of the amplification target circle is accomplished by cleaving the cleavable bond.
15. (Original) The method of claim 14, wherein the cleavable bond is cleaved by treatment with a reducing agent.
16. (Original) The method of claim 15, wherein the cleavable bond is a disulfide bond.

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

17. (Original) The method of claim 16, wherein the circle linker comprises dithiobis succinimidyl propionate, dimethyl 3,3'-dithiobispropionimidate, dithio-bis-maleimidoethane, 3,3'-dithiobis sulfosuccinimidyl propionate, succinimidyl 6-[3-(2-pyridyldithio)-propionamido]hexonate, or N-succinimidyl 3-[2-pyridyldithio]propionate.

18. (Original) The method of claim 14, wherein the cleavable bond is cleaved by treatment with periodate.

19. (Original) The method of claim 18, wherein the cleavable bond is a dihydroxy bond.

20. (Original) The method of claim 19, wherein the circle linker comprises 1,4 bis-maleimidyl-2,3-dihydroxybutane, disuccinimidyl tartrate, or disulfosuccinimidyl tartrate.

21. (Original) The method of claim 12, wherein the circle linker is coupled to the amplification target circle via a reactive group on the amplification target circle.

22. (Original) The method of claim 21, wherein the reactive group is an allyl amino group.

23. (Original) The method of claim 1, wherein a plurality of reporter binding molecules are brought into contact with the one or more analyte samples.

24. (Original) The method of claim 1, wherein a plurality of analyte samples are brought into contact with the one or more reporter binding molecules.

25. (Original) The method of claim 1, wherein at least one of the analytes is a protein or peptide.

26. (Original) The method of claim 1, wherein at least one of the analytes is a lipid, glycolipid, or proteoglycan.

27. (Original) The method of claim 1, wherein at least one of the analytes is from a human source.

28. (Original) The method of claim 1, wherein at least one of the analytes is from a non-human source.

29. (Original) The method of claim 1, wherein none of the analytes are nucleic acids.

30. (Original) The method of claim 1, wherein the specific binding molecules that interact with the analytes are separated by

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

bringing into contact at least one of the analyte samples and one or more analyte capture agents, wherein each analyte capture agent interacts with an analyte directly or indirectly, wherein at least one analyte, if present in the analyte sample, interacts with at least one analyte capture agent, and

separating analyte capture agents from the analyte samples, thus separating specific binding molecules that interact with the analytes from the analyte samples.

31. (Original) The method of claim 30, wherein at least one analyte capture agent is associated with a solid support, wherein analytes that interact with the analyte capture agent associated with a solid support become associated with the solid support.

32. (Original) The method of claim 31, wherein the solid support comprise one or more reaction chambers, wherein a plurality of the analyte capture agents are located in the same reaction chamber on the solid support.

33. (Original) The method of claim 31, wherein a plurality of reporter binding molecules are brought into contact with one or more analyte samples, wherein two or more of the amplification target circles are replicated in the same reaction chamber of the solid support, wherein the amplification target circles replicated in the same reaction chamber of the solid support are different, wherein each different amplification target circle produces a different tandem sequence DNA,

wherein the presence or absence of different analytes is indicated by the presence or absence of corresponding tandem sequence DNA.

34. (Original) The method of claim 33, wherein replication of each different amplification target circle is primed by a different one of the rolling circle replication primers.

35. (Original) The method of claim 31, wherein the solid support comprises acrylamide, agarose, cellulose, cellulose, nitrocellulose, glass, gold, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, functionalized silane, polypropylfumarate, collagen, glycosaminoglycans, or polyamino acids.

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

36. (Original) The method of claim 30, further comprising bringing into contact at least one of the analyte samples and at least one of the reporter binding molecules with at least one accessory molecule, wherein the accessory molecule affects the interaction of at least one of the analytes and at least one of the specific binding molecules or at least one of the analyte capture agents.

37. (Original) The method of claim 36, wherein the accessory molecule is brought into contact with at least one of the analyte samples, at least one of the reporter binding molecules, or both, prior to, simultaneous with, or following step (a).

38. (Original) The method of claim 36, wherein at least one analyte capture agent is associated with a solid support, wherein the accessory molecule is associated with the solid support.

39. (Original) The method of claim 38, wherein the accessory molecule is associated with the solid support by bringing the accessory molecule into contact with the solid support prior to, simultaneous with, or following step (a).

40. (Original) The method of claim 36, wherein the accessory molecule is a protein kinase, a protein phosphatase, an enzyme, or a compound.

41. (Original) The method of claim 36, wherein the accessory molecule is a molecule of interest, wherein one or more of the analytes are test molecules, wherein interactions of the test molecules with the molecule of interest are detected.

42. (Original) The method of claim 36, wherein at least one of the analytes is a molecule of interest, wherein the accessory molecule is a test molecule, wherein interactions of the test molecule with the molecule of interest are detected.

43. (Original) The method of claim 30, wherein the analyte samples include one or more first analyte samples and one or more second analyte samples, wherein the reporter binding molecules include one or more first reporter binding molecules and one or more second reporter binding molecules,

the method further comprising, following step (a) and prior to bringing the analyte samples and the solid support into contact,

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

mixing one or more of the first analyte samples and one or more of the second analyte samples,

wherein for each first reporter binding molecule there is a matching second reporter binding molecule, wherein the specific binding molecules of the first reporter binding molecules interacts with the same analyte as the specific binding molecules of the matching second reporter binding molecule,

wherein the amplification target circle of each different reporter binding molecule is different, wherein each different amplification target circle produces a different tandem sequence DNA,

wherein the presence or absence of the same analyte in different analyte samples is indicated by the presence or absence of corresponding tandem sequence DNA.

44. (Original) The method of claim 43, wherein replication of each different amplification target circle is primed by a different one of the rolling circle replication primers.

45. (Original) The method of claim 44, wherein the tandem sequence DNA corresponding to one of the analytes and produced in association with a first reporter binding molecule is in the same location on the solid support as tandem sequence DNA corresponding to the same analyte and produced in association with the matching second reporter binding molecule,

wherein the presence or absence of the same analyte in different analyte samples is indicated by the presence or absence of corresponding tandem sequence DNA.

46. (Original) The method of claim 30, wherein at least one of the analyte capture agents is a molecule of interest, wherein one or more of the analytes are test molecules, wherein interactions of the test molecules with the molecule of interest are detected.

47. (Original) The method of claim 30, wherein at least one of the analytes is a molecule of interest, wherein one or more of the analyte capture agents are test molecules, wherein interactions of the test molecules with the molecule of interest are detected.

48. (Original) The method of claim 1, wherein a plurality of reporter binding molecules are brought into contact with one or more analyte samples, wherein two or more of the amplification target circles are replicated in the same reaction, wherein the amplification target

circles replicated in the same reaction are different, wherein each different amplification target circle produces a different tandem sequence DNA,

wherein the presence or absence of different analytes is indicated by the presence or absence of corresponding tandem sequence DNA.

49. (Original) The method of claim 48, wherein replication of each different amplification target circle is primed by a different one of the rolling circle replication primers.

50. (Original) The method of claim 1, further comprising, prior to, simultaneous with, or following step (a),

bringing into contact one or more first analyte capture agents and one or more first analyte samples, and bringing into contact one or more second analyte capture agents and one or more second analyte samples,

wherein each analyte capture agent comprises an analyte interaction portion and a capture portion, wherein for each first analyte capture agent there is a matching second analyte capture agent,

wherein the analyte interaction portions of the first analyte capture agents interact with the same analyte as the analyte interaction portions of the matching second analyte capture agents,

wherein the capture portions of the first and second analyte capture agents each interact with a specific binding molecule of one or more of the reporter binding molecules, wherein the capture portions of the first analyte capture agents interact with different specific binding molecules than the capture portions of the matching second analyte capture agents,

wherein each different specific binding molecule is part of a different one of the reporter binding molecules, wherein the amplification target circle of each different reporter binding molecule is different, wherein replication of each different amplification target circle is primed by a different one of the rolling circle replication primers, wherein each different amplification target circle produces a different tandem sequence DNA, wherein the amplification target circle of a reporter binding molecule that comprises a specific binding molecule that interacts with an analyte capture agent corresponds to the analyte capture agent,

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

wherein the presence or absence of the same analyte in different analyte samples is indicated by the presence or absence of corresponding tandem sequence DNA.

51. (Original) The method of claim 50, further comprising mixing one or more of the first analyte samples and one or more of the second analyte samples.

52. (Original) The method of claim 50, further comprising mixing the one or more first analyte capture agents and the one or more second analyte capture agents.

53. (Original) The method of claim 52, wherein mixing the one or more first analyte capture agents and the one or more second analyte capture agents is accomplished by associating, simultaneously or sequentially, the one or more first analyte capture agents and the one or more second analyte capture agents with the same solid support.

54. (Original) The method of claim 50, wherein the tandem sequence DNA corresponding to one of the analytes and produced in association with a first analyte capture agent is in the same location as, and is simultaneously detected with, tandem sequence DNA corresponding to the same analyte and produced in association with the matching second analyte capture agent,

wherein the presence or absence of the same analyte in different analyte samples is indicated by the presence or absence of corresponding tandem sequence DNA.

55. (Original) The method of claim 50, wherein the capture portion of each first analyte capture agent is the same, wherein the reporter binding molecules corresponding to the first analyte capture agents are the same, wherein the amplification target circles corresponding to the first analyte capture agents are the same,

wherein the capture portion of each second analyte capture agent is the same, wherein the reporter binding molecules corresponding to the second analyte capture agents are the same, wherein the amplification target circles corresponding to the second analyte capture agents are the same.

56. (Original) The method of claim 1, wherein at least one of the specific binding molecules is an antibody specific for at least one of the analytes.

57. (Original) The method of claim 1, wherein at least one of the specific binding molecules is a molecule that specifically binds to at least one of the analytes.

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

58. (Original) The method of claim 1, wherein at least one of the specific binding molecules is a molecule that specifically binds to at least one of the analytes in combination with an accessory molecule.

59. (Original) The method of claim 1, wherein the specific binding molecules and analytes interact by binding to each other directly or indirectly.

60. (Original) The method of claim 1, wherein at least one accessory molecule is brought into contact with at least one of the analyte samples and at least one of the reporter binding molecules, wherein the accessory molecule affects the interaction of at least one of the analytes and at least one of the specific binding molecules or at least one of the analyte capture agents.

61. (Original) The method of claim 60, wherein the accessory molecule competes with the interaction of at least one of the specific binding molecules or at least one of the analyte capture agents.

62. (Original) The method of claim 61, wherein the accessory molecule is an analog of at least one of the analytes.

63. (Original) The method of claim 60, wherein the accessory molecule facilitates the interaction of at least one of the specific binding molecules or at least one of the analyte capture agents.

64. (Original) The method of claim 60, wherein the accessory molecule is brought into contact with at least one of the analyte samples, at least one of the reporter binding molecules, or both, prior to, simultaneous with, or following step (a).

65. (Original) The method of claim 60, wherein the accessory molecule is a protein kinase, a protein phosphatase, an enzyme, or a compound.

66. (Original) The method of claim 60, wherein the accessory molecule is at least 20% pure.

67. (Original) The method of claim 60, wherein the accessory molecule is at least 50% pure.

68. (Original) The method of claim 60, wherein the accessory molecule is at least 80% pure.

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

69. (Original) The method of claim 60, wherein the accessory molecule is at least 90% pure.

70. (Original) The method of claim 1, wherein at least one of the analytes is associated with a solid support.

71. (Original) The method of claim 70, wherein the solid support comprises one or more reaction chambers, wherein a plurality of the analytes associated with the solid support are associated with the solid support in the same reaction chamber.

72. (Original) The method of claim 70, wherein at least one of the analytes associated with the solid support is associated with the solid support indirectly.

73. (Original) The method of claim 72, wherein the analytes associated with the solid support interact with analyte capture agents, and wherein the analyte capture agents are associated with the solid support thereby indirectly associating the analytes with the solid support.

74. (Original) The method of claim 1, wherein at least one specific binding molecule interacts with at least one analyte indirectly.

75. (Original) The method of claim 74, wherein the analyte interacts with an analyte capture agent, and wherein the specific binding molecule interacts with the analyte capture agent thereby indirectly associating the specific binding molecule with the analyte.

76. (Original) The method of claim 1, wherein at least one of the analytes is a modified form of another analyte, wherein the specific binding molecule of at least one of the reporter binding molecules interacts, directly or indirectly, with the analyte that is a modified form of the other analyte, and wherein the specific binding molecule of another reporter binding molecule interacts, directly or indirectly, with the other analyte.

77. (Original) The method of claim 76, wherein the analytes are proteins, wherein the modification of the modified form of the other analyte is a post-translational modification.

78. (Original) The method of claim 77, wherein the modification is phosphorylation or glycosylation.

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

79. (Original) The method of claim 1, wherein detection of the tandem sequence DNA is accomplished by

mixing a set of detection probes with the tandem sequence DNA under conditions that promote hybridization between the tandem sequence DNA and the detection probes.

80. (Original) The method of claim 79, wherein a plurality of different tandem sequence DNAs are detected separately and simultaneously via multiplex detection.

81. (Original) The method of claim 80, wherein the set of detection probes is labeled using combinatorial multicolor coding.

82. (Original) The method of claim 1, further comprising, simultaneous with, or following, step (d),

bringing into contact a secondary DNA strand displacement primer and the tandem sequence DNA, and incubating under conditions that promote (i) hybridization between the tandem sequence DNA and the secondary DNA strand displacement primer, and (ii) replication of the tandem sequence DNA, wherein replication of the tandem sequence DNA results in the formation of secondary tandem sequence DNA.

83. (Original) The method of claim 82, wherein the tandem sequence DNA, secondary tandem sequence DNA, or both, are detected during replication of the amplification target circles.

84. (Original) The method of claim 83, wherein the tandem sequence DNA, secondary tandem sequence DNA, or both, are detected by detecting fluorescent moieties incorporated into the tandem sequence DNA, secondary tandem sequence DNA, or both.

85. (Original) The method of claim 82, wherein the tandem sequence DNA, secondary tandem sequence DNA, or both, are detected during replication of the tandem sequence DNA.

86. (Original) The method of claim 82, wherein at least one of the rolling circle replication primers is a fluorescent quenched primer.

87. (Original) The method of claim 82, wherein at least one of the secondary DNA strand displacement primers is a fluorescent quenched primer.

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

88. (Original) The method of claim 82, wherein at least one of the rolling circle replication primers and at least one of the secondary DNA strand displacement primers are fluorescent quenched primers.

89. (Original) The method of claim 82, wherein the tandem sequence DNA, secondary tandem sequence DNA, or both, are detected by detecting fluorescent moieties incorporated into the tandem sequence DNA.

90. (Original) The method of claim 82, wherein the secondary tandem sequence DNA is replicated to form higher order tandem sequence DNA.

91. (Original) The method of claim 90, wherein the amplification target circles, the tandem sequence DNA, and the secondary tandem sequence DNA are replicated simultaneously.

92. (Original) The method of claim 90, wherein the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination, is detected during replication of the amplification target circles.

93. (Original) The method of claim 90, wherein the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination, is detected by detecting fluorescent moieties incorporated into the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination.

94. (Original) The method of claim 90, wherein the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination, is detected during replication of the tandem sequence DNA.

95. (Original) The method of claim 90, wherein at least one of the rolling circle replication primers is a fluorescent quenched primer.

96. (Original) The method of claim 90, wherein at least one of the secondary DNA strand displacement primers is a fluorescent quenched primer.

97. (Original) The method of claim 90, wherein at least one of the rolling circle replication primers and at least one of the secondary DNA strand displacement primers are fluorescent quenched primers.

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

98. (Original) The method of claim 90, wherein the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination, is detected by detecting fluorescent moieties incorporated into the tandem sequence DNA.

99. (Original) The method of claim 1, wherein the tandem sequence DNA is detected during replication of the amplification target circles.

100. (Original) The method of claim 99, wherein the tandem sequence DNA is detected by detecting fluorescent moieties incorporated into the tandem sequence DNA.

101. (Original) The method of claim 1, wherein at least one of the rolling circle replication primers is a fluorescent quenched primer.

102. (Original) The method of claim 1, wherein the tandem sequence DNA is detected by detecting fluorescent moieties incorporated into the tandem sequence DNA.

103. (Original) The method of claim 1, wherein the reporter binding molecules are at least 20% pure.

104. (Original) The method of claim 1, wherein the reporter binding molecules are at least 50% pure.

105. (Original) The method of claim 1, wherein the reporter binding molecules are at least 80% pure.

106. (Original) The method of claim 1, wherein the reporter binding molecules are at least 90% pure.

107. (Currently Amended) A method for detecting one or more analytes, the method comprising

(a) bringing into contact one or more analyte samples and one or more analyte capture agents, wherein each analyte capture agent interacts with an analyte directly or indirectly, wherein at least one analyte, if present in the analyte sample, interacts with at least one analyte capture agent,

incubating the analyte samples and the analyte capture agents under conditions that promote interaction of the analyte capture agents and analytes,

(b) bringing into contact at least one of the analyte samples and one or more reporter binding molecules, wherein each reporter binding molecule comprises a specific binding

molecule and an amplification target circle, wherein each specific binding molecule interacts with an analyte capture agent directly or indirectly,

incubating the analyte samples and the reporter binding molecules under conditions that promote interaction of the specific binding molecules and analyte capture agents, and

separating the specific binding molecules that interact with the analyte capture agents from the specific binding molecules that do not interact with the analyte capture agents,

(c) decoupling the amplification target circles associated with the analytes from the ~~reporter binding molecules~~ specific binding molecules that interact with the analytes,

(d) bringing into contact the amplification target circles and one or more rolling circle replication primers, wherein the amplification target circles each comprise a single-stranded, circular DNA molecule comprising a primer complement portion, wherein the primer complement portion is complementary to at least one of the rolling circle replication primers, and

incubating the rolling circle replication primers and amplification target circles under conditions that promote hybridization between the amplification target circles and the rolling circle replication primers,

(e) incubating the rolling circle replication primers and amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA, wherein detection of tandem sequence DNA indicates the presence of the corresponding analytes.

108. (Currently Amended) A method for detecting one or more analytes, the method comprising

(a) treating one or more analyte samples so that one or more analytes are modified,

(b) bringing into contact at least one of the analyte samples and one or more reporter binding molecules, wherein each reporter binding molecule comprises a specific binding molecule and an amplification target circle, wherein each specific binding molecule interacts with a modified analyte directly or indirectly,

incubating the analyte samples and the reporter binding molecules under conditions that promote interaction of the specific binding molecules and modified analytes, and

separating the specific binding molecules that interact with the modified analytes from the specific binding molecules that do not interact with the modified analytes,

(c) decoupling the amplification target circles associated with the analytes from the ~~reporter binding molecules~~ specific binding molecules that interact with the analytes,

(d) bringing into contact the amplification target circles and one or more rolling circle replication primers, wherein the amplification target circles each comprise a single-stranded, circular DNA molecule comprising a primer complement portion, wherein the primer complement portion is complementary to at least one of the rolling circle replication primers, and

incubating the rolling circle replication primers and amplification target circles under conditions that promote hybridization between the amplification target circles and the rolling circle replication primers,

(e) incubating the rolling circle replication primers and amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA, wherein detection of tandem sequence DNA indicates the presence of the corresponding modified analytes.

109. (Original) The method of claim 108, wherein all of the analytes are modified by associating a modifying group to the analytes, wherein the modifying group is the same for all of the analytes, wherein all of the specific binding molecules interact with the modifying group.

110. (Currently Amended) A method for detecting one or more analytes, the method comprising

(a) bringing into contact one or more analyte samples and a set of analyte capture agents, a set of accessory molecules, or both, wherein each analyte capture agent interacts with an analyte directly or indirectly,

(b) prior to, simultaneous with, or following step (a), bringing into contact at least one of the analyte samples and one or more reporter binding molecules, wherein each reporter binding molecule comprises a specific binding molecule and an amplification target circle, wherein each specific binding molecule interacts with an analyte directly or indirectly, wherein each accessory

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

molecule affects the interaction of at least one of the analytes and at least one of the specific binding molecules or at least one of the analyte capture agents,

(c) simultaneous with, or following, steps (a) and (b), incubating the analyte samples, the analyte capture agents, the accessory molecules, and the reporter binding molecules under conditions that promote interaction of the specific binding molecules, analytes, analyte capture agents, and accessory molecules, and

separating the specific binding molecules that interact with the analytes from the specific binding molecules that do not interact with the analytes,

decoupling the amplification target circles associated with the analytes from the ~~reporter binding molecules~~ specific binding molecules that interact with the analytes,

(d) bringing into contact the amplification target circles and one or more rolling circle replication primers, wherein the amplification target circles each comprise a single-stranded, circular DNA molecule comprising a primer complement portion, wherein the primer complement portion is complementary to at least one of the rolling circle replication primers, and

incubating the rolling circle replication primers and amplification target circles under conditions that promote hybridization between the amplification target circles and the rolling circle replication primers,

(e) incubating the reporter binding molecules and amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA, wherein detection of tandem sequence DNA indicates the presence of the corresponding analytes.

111. (Original) The method of claim 110, wherein the analyte capture agents are immobilized on a solid support, wherein the solid support comprises one or more reaction chambers, wherein a plurality of the analyte capture agents are immobilized in the same reaction chamber of the solid support.

112. (Original) The method of claim 111, wherein the analyte capture agents are immobilized to the solid support at a density exceeding 400 different analyte capture agents per cubic centimeter.

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

113. (Original) The method of claim 111, wherein the analyte capture agents are peptides.

114. (Original) The method of claim 113, wherein each of the different peptides is at least 4 amino acids in length.

115. (Original) The method of claim 114, wherein each different peptide is from about 4 to about 20 amino acids in length.

116. (Original) The method of claim 114, wherein each different peptide is at least 10 amino acids in length.

117. (Original) The method of claim 114, wherein each different peptide is at least 20 amino acids in length.

118. (Original) The method of claim 111, wherein the solid support comprises a plurality of reaction chambers.

119. (Original) The method of claim 111, wherein the solid support comprises acrylamide, agarose, cellulose, cellulose, nitrocellulose, glass, gold, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, functionalized silane, polypropylfumarate, collagen, glycosaminoglycans, or polyamino acids.

120. (Original) The method of claim 111, wherein the analyte capture agents in the reaction chambers are at least 20% pure.

121. (Original) The method of claim 111, wherein the analyte capture agents in the reaction chambers are at least 50% pure.

122. (Original) The method of claim 111, wherein the analyte capture agents in the reaction chambers are at least 80% pure.

123. (Original) The method of claim 111, wherein the analyte capture agents in the reaction chambers are at least 90% pure.

124. (Currently Amended) A method for detecting one or more analytes, the method comprising

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

bringing into contact one or more analyte samples and one or more reporter binding molecules, wherein each reporter binding molecule comprises a specific binding molecule and an amplification target circle, wherein each specific binding molecule can interact with an analyte directly or indirectly,

separating the specific binding molecules that interact with the analytes from the specific binding molecules that do not interact with the analytes,

decoupling the amplification target circles associated with the analytes from the ~~reporter binding molecules~~ specific binding molecules that interact with the analytes,

replicating the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA, secondary tandem sequence DNA, and higher order tandem sequence DNA, wherein detection of tandem sequence DNA, secondary tandem sequence DNA, and higher order tandem sequence DNA, or a combination, indicates the presence of the corresponding analytes.

125. (Original) The method of claim 124, wherein the amplification target circles, the tandem sequence DNA, and the secondary tandem sequence DNA are replicated simultaneously.

126. (Original) The method of claim 124, wherein the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination, is detected during replication of the amplification target circles.

127. (Original) The method of claim 124, wherein the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination, is detected by detecting fluorescent moieties incorporated into the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination.

128. (Original) The method of claim 124, wherein the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination, is detected during replication of the tandem sequence DNA.

129. (Original) The method of claim 124, wherein at least one of the rolling circle replication primers is a fluorescent quenched primer.

130. (Original) The method of claim 124, wherein at least one of the secondary DNA strand displacement primers is a fluorescent quenched primer.

ATTORNEY DOCKET NO. 13172.0015U1
Application No. 10/072,666

131. (Original) The method of claim 124, wherein at least one of the rolling circle replication primers and at least one of the secondary DNA strand displacement primers are fluorescent quenched primers.

132. (Original) The method of claim 124, wherein the tandem sequence DNA, secondary tandem sequence DNA, higher order tandem sequence DNA, or a combination, is detected by detecting fluorescent moieties incorporated into the tandem sequence DNA.

133. (Currently Amended) A method for detecting one or more analytes, the method comprising

bringing into contact one or more analyte samples and one or more reporter binding molecules, wherein each reporter binding molecule comprises a specific binding molecule and an amplification target circle, wherein each specific binding molecule can interact with an analyte directly or indirectly,

separating the specific binding molecules that interact with the analytes from the specific binding molecules that do not interact with the analytes,

decoupling the amplification target circles associated with the analytes from the ~~reporter binding molecules~~ specific binding molecules that interact with the analytes,

replicating the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA, wherein detection of tandem sequence DNA indicates the presence of the corresponding analytes.

134. (Currently Amended) A method for detecting one or more analytes, the method comprising

bringing into contact one or more analyte samples and one or more analyte capture agents, wherein each analyte capture agents can interact with an analyte directly or indirectly,

bringing into contact at least one of the analyte samples and one or more reporter binding molecules, wherein each reporter binding molecule comprises a specific binding molecule and an amplification target circle, wherein each specific binding molecule can interact with an analyte capture agent directly or indirectly,

separating the specific binding molecules that interact with the analyte capture agents from the specific binding molecules that do not interact with the analyte capture agents,

decoupling the amplification target circles associated with the analytes from the ~~reporter binding molecules~~ specific binding molecules that interact with the analytes,

replicating the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA, wherein detection of tandem sequence DNA indicates the presence of the corresponding analytes.

135. (Currently Amended) A method for detecting one or more analytes, the method comprising

treating one or more analyte samples so that one or more analytes are modified,

bringing into contact at least one analyte sample and one or more reporter binding molecules, wherein each reporter binding molecule comprises a specific binding molecule and an amplification target circle, wherein each specific binding molecule can interact with a modified analyte directly or indirectly,

separating the specific binding molecules that interact with the modified analytes from the specific binding molecules that do not interact with the modified analytes,

decoupling the amplification target circles associated with the analytes from the ~~reporter binding molecules~~ specific binding molecules that interact with the analytes,

replicating the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA, wherein detection of tandem sequence DNA indicates the presence of the corresponding modified analytes.

136. (Currently Amended) A method for detecting one or more analytes, the method comprising

bringing into contact one or more analyte samples and a set of analyte capture agents, a set of accessory molecules, or both, wherein each analyte capture agent can interact with an analyte directly or indirectly,

bringing into contact at least one of the analyte samples and one or more reporter binding molecules, wherein each reporter binding molecule comprises a specific binding molecule and an amplification target circle, wherein each specific binding molecule can interact with an analyte directly or indirectly, wherein each accessory molecule can affect the interaction of at least one of the analytes and at least one of the specific binding molecules or at least one of the analyte capture agents,

separating the specific binding molecules that interact with the analytes from the specific binding molecules that do not interact with the analytes,

decoupling the amplification target circles associated with the analytes from the ~~reporter binding molecules~~ specific binding molecules that interact with the analytes,

replicating the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA, wherein detection of tandem sequence DNA indicates the presence of the corresponding analytes.

137. (Withdrawn) A kit comprising

(a) a plurality of reporter binding molecules, wherein each reporter binding molecule comprises a specific binding molecule and an amplification target circle, wherein the amplification target circle can be decoupled from the reporter binding molecule, wherein each specific binding molecule interacts with an analyte directly or indirectly, and

(b) a plurality of analyte capture agents, wherein each analyte capture agent interacts with an analyte directly or indirectly.

138. (Withdrawn) The kit of claim 137, wherein the analyte capture agents are associated with a solid support.